



Attorney's Docket No.: 13498-010003

THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Zhaoda Zhang et al.

Art Unit : Unknown

Serial No. : Not Assigned

Examiner : Unknown

Filed : February 25, 2004

Title : PEPTIDE-BASED MULTIMERIC TARGETED CONTRAST AGENTS

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as indicated on the following pages.

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February 25, 2004  
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Applicant : Zhaoda Zhang et al.  
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Page : 2 of 25

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Amendments to the Specification:

Please replace the paragraph beginning at page 1 after the title with the following amended paragraph:

**CROSS-REFERENCE TO RELATED APPLICATIONS**

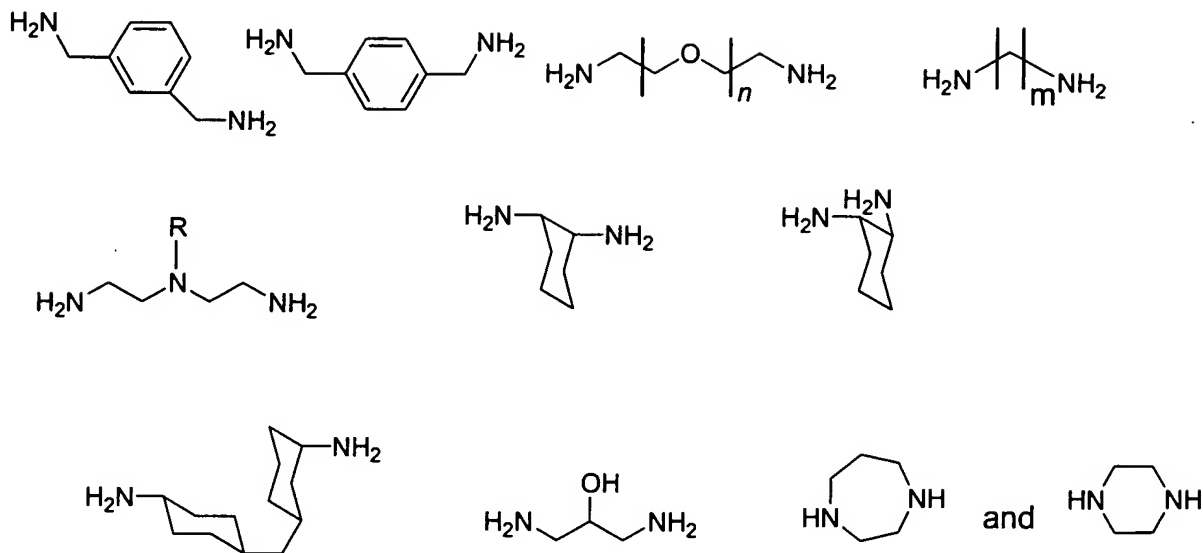
This application is a divisional of and claims priority to U.S. Application No. 10/209,183, filed July 30, 2002, and is related to U.S. Application No. 10/209,172, filed July 30, 2002, both of which claim ~~claims~~ priority from U.S. Provisional Application No. 60/308,721, filed July 30, 2001, all of which are incorporated herein in their entirety.

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (original) A method of making an MR imaging agent, said method comprising:
  - a) reacting a peptide having an N-terminal amine functional group with a linker-subunit moiety to form a modified peptide having a C-terminal amine functional group and said N-terminal amine functional group;
  - b) covalently attaching a linker moiety to the C-terminal amine functional group and to the N-terminal amine functional group to form a precursor MR imaging agent; and
  - c) converting the precursor MR imaging agent to the MR imaging agent.
2. (currently amended) The method of claim 1, wherein the linker-subunit moiety is ~~selected from the group consisting of:~~



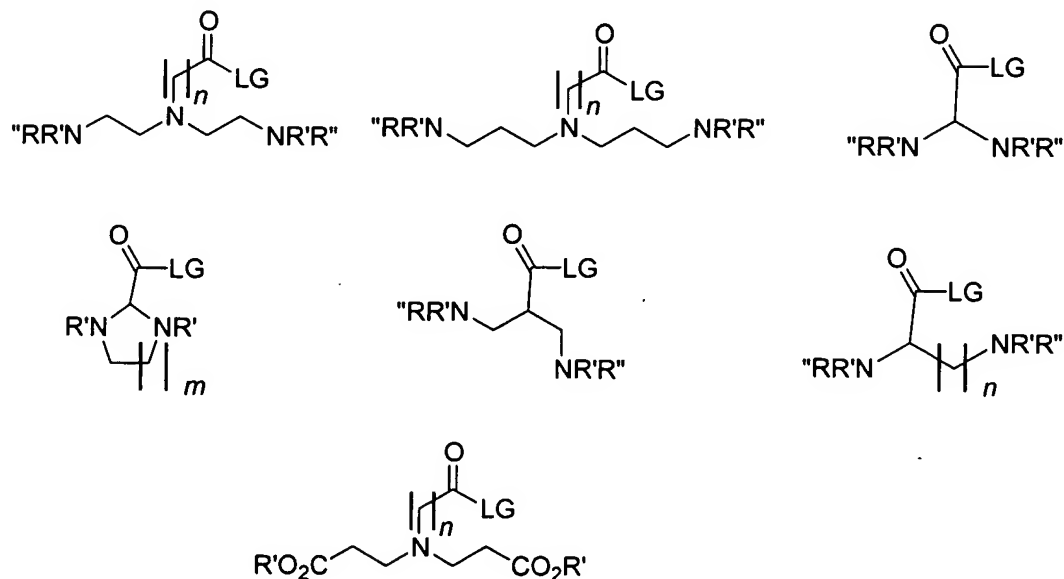
wherein:

~~n is an integer from 1 to 4;~~

~~m is an integer selected 1 to 12; and~~

~~R is an aliphatic or aromatic group.~~

3. (original) The method of claim 1, wherein the linker moiety is selected from the group consisting of



wherein:

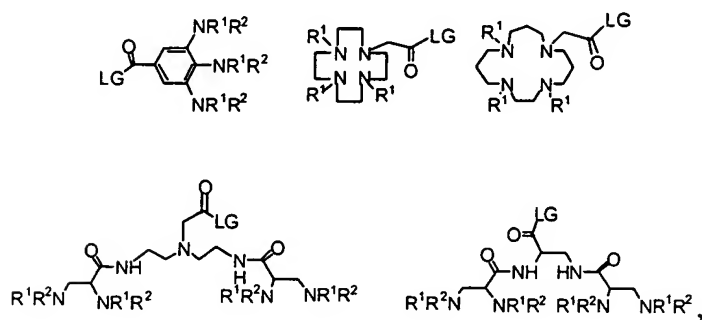
m is an integer from 1 to 4;

n is an integer from 0 to 4;

LG is a leaving group; and

R' and R'' independently are selected from the group consisting of hydrogen and a chemical protecting group.

4. (original) The method according to claim 1, wherein the linker moiety is selected from the group consisting of:



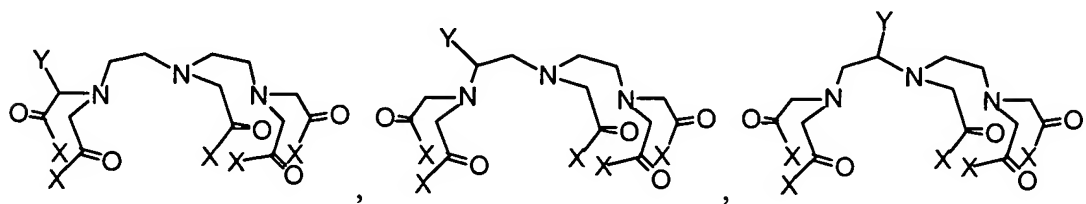
wherein;

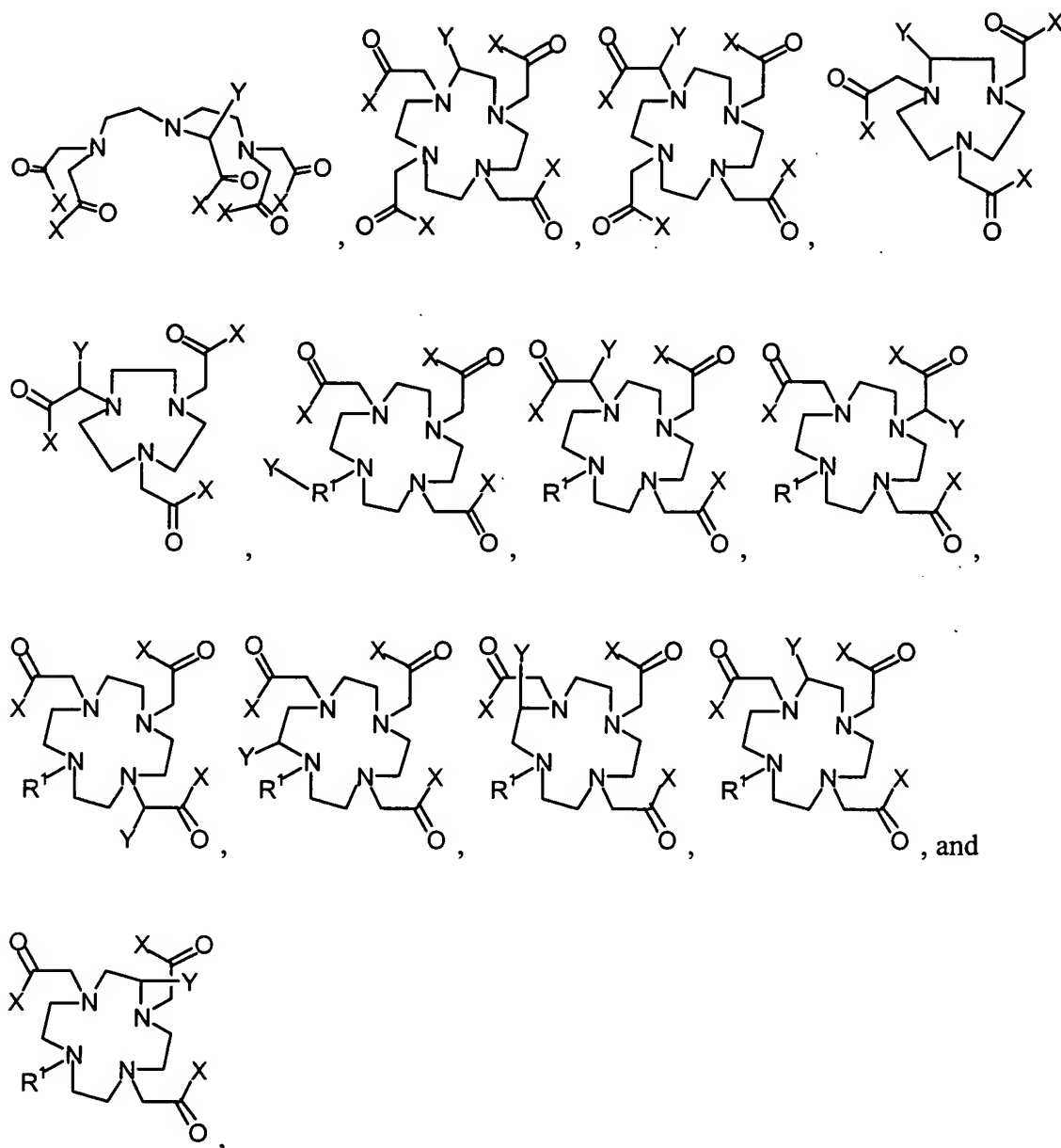
LG is a leaving group; and

R¹ and R² independently are selected from the group consisting of hydrogen and a chemical protecting group.

5. (original) The method of claim 3 or claim 4, wherein the LG is selected from the group consisting of -OH, activated ester, halide, and anhydride, and wherein the chemical protecting group is selected from the group consisting of Boc, Fmoc, CBZ, t-butyl, benzyl, and allyl.

6. (original) The method of claim 5, wherein the activated ester is selected from the group consisting of pentafluorophenol (Pfp), N-hydroxysuccinimide (NHS), N-Hydroxysulfosuccinimide Sodium Salt (NHSS), 2-Thioxothiazolidin-1-yl, and hydroxybenzotriazole (OBT).
7. (original) The method of claim 5, wherein the halide is selected from the group consisting of F, Cl, Br, and I.
8. (original) The method of claim 1, wherein converting the precursor MR imaging agent to the MR imaging agent comprises:
- (a) reacting the precursor imaging agent with a precursor chelate moiety to form a covalent bond between the precursor chelate moiety and the linker moiety of the precursor MR imaging agent, the precursor chelate moiety comprising a plurality of carboxylate precursor groups, the carboxylate precursor groups capable of being transformed into carboxylate moieties;
  - (b) transforming a plurality of the carboxylate precursor groups of the bound precursor chelate moiety to a plurality of carboxylate moieties, the carboxylate moieties capable of complexing a paramagnetic metal ion; and
  - (c) complexing a paramagnetic metal ion to the plurality of carboxylate moieties to produce the MR imaging agent.
9. (original) The method of claim 8, wherein the precursor chelate moiety is selected from the group consisting of:

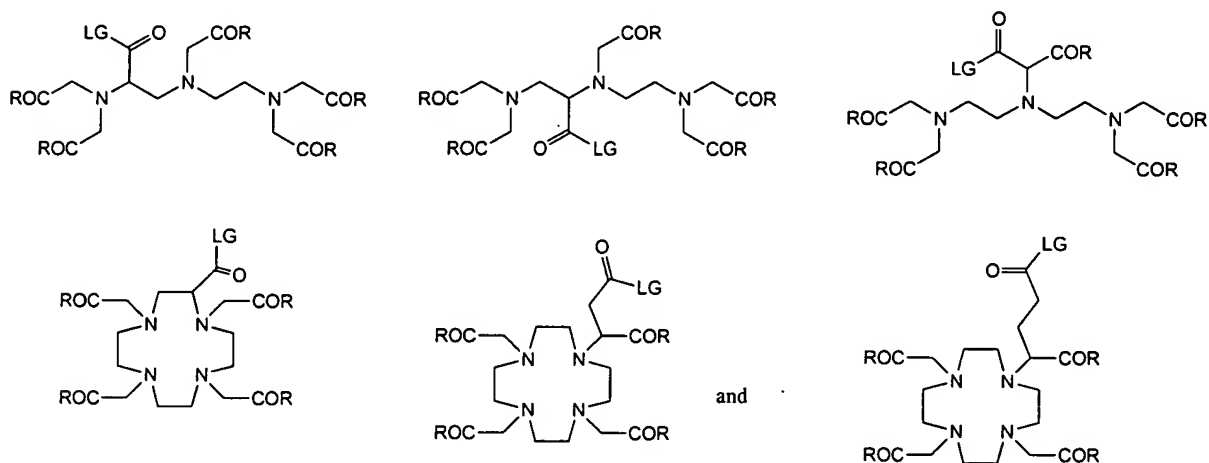




wherein Y is a synthetic moiety capable of forming a covalent bond with the attached linker moiety, and wherein each X, independently, is an O<sup>-</sup> or an O<sup>-</sup> precursor so that X, upon conversion to O<sup>-</sup>, is capable of forming a carboxylate moiety with its adjacent carbonyl, and R<sup>1</sup> is an uncharged chemical moiety, an aliphatic, alkyl group, or cycloalkyl group, or uncharged substituted versions thereof.

10. (original) The method of claim 9, wherein the synthetic moiety is selected from the group consisting of a carboxylic acid, activated ester, acid halide, anhydride, alkyl halide, isocyanate, and isothiocyanate, and wherein the O<sup>-</sup> precursor is selected from the group consisting of -OH, -OMe, OEt, OtBu, Obenzyl, and O-allyl.

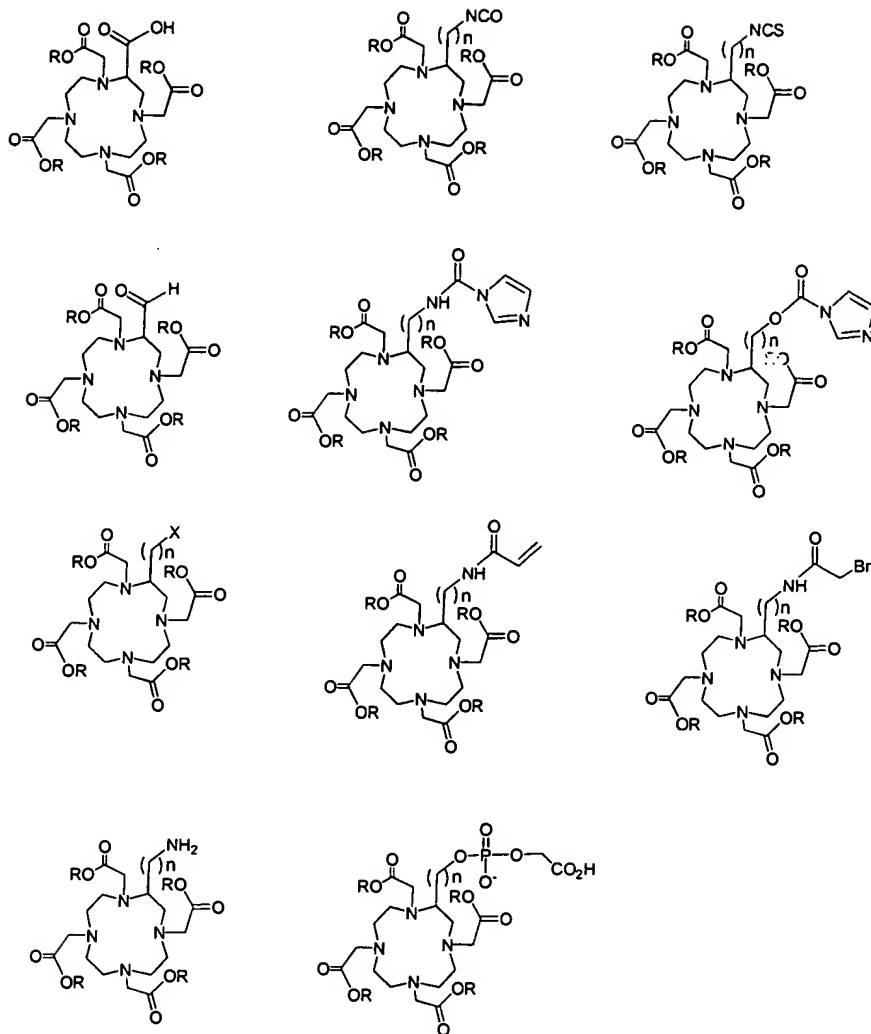
11. (original) The method of claim 8, wherein the precursor chelate moiety is selected from the group consisting of:



wherein LG is a leaving group selected from the group consisting of -OH, activated ester, halide, and anhydride, and wherein each R, independently, is an O<sup>-</sup> or an O<sup>-</sup> precursor selected from the group consisting of OH, -O-Me, O-Et, O-tBu, O-benzyl, and O-allyl, so that R, upon conversion to O<sup>-</sup>, is capable of forming a carboxylate moiety with its adjacent carbonyl.

12. (original) The method of claim 8, wherein the precursor chelate moiety is selected from the group consisting of:





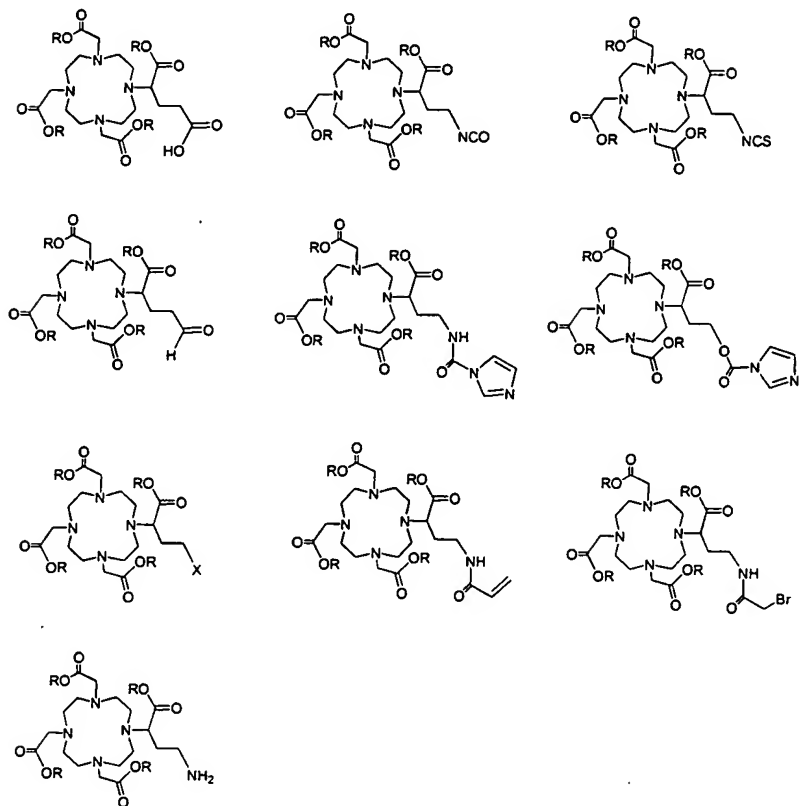
wherein:

$n$  is an integer from 1 to 4;

$R$  is selected from the group consisting of a negative charge and a negative charge precursor capable of being transformed into a negative charge; and

$X$  is a chemical leaving group selected from the group consisting of -Cl, -Br, -I, -MsO, -TsO, and -TfO.

13. (original) The method of claim 8, wherein the precursor chelate moiety is selected from the group consisting of:



wherein:

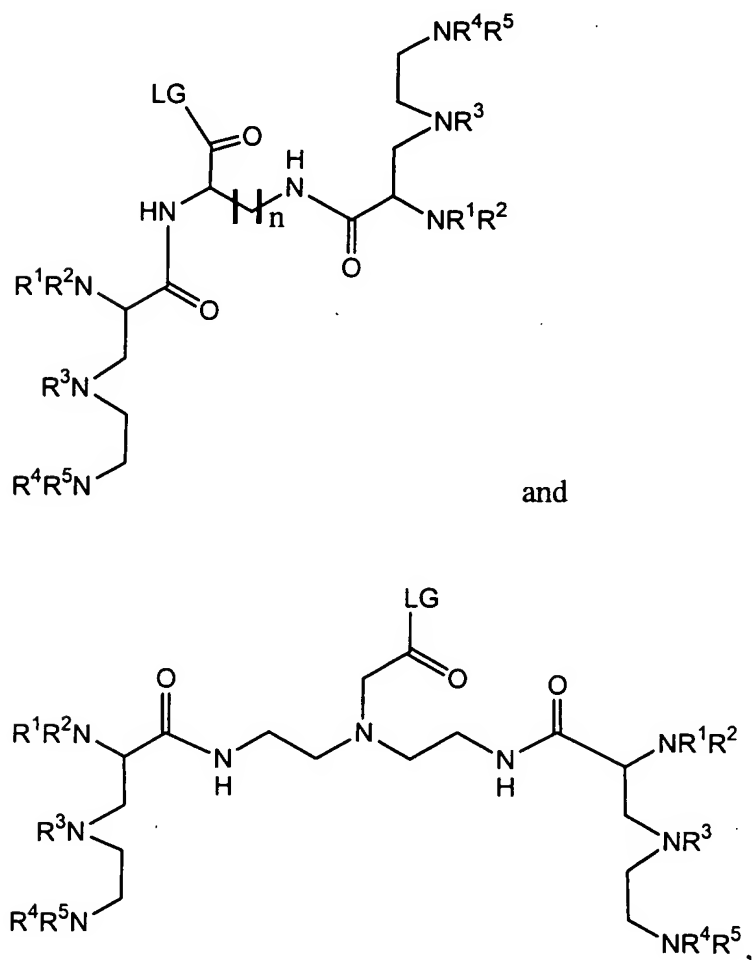
R is selected from the group consisting of a negative charge and a negative charge precursor capable of being transformed into a negative charge; and

X is a chemical leaving group selected from the group consisting of -Cl, -Br, -I, -MsO, -TsO, and -TfO.

14. (original) The method of claim 12 or 13, wherein the negative charge precursor is selected from the group consisting of -H, -Me, -Et, -t-Bu, -benzyl, and -allyl.

15. (original) The method of claim 1, wherein the linker moiety is covalently conjugated to a precursor chelate moiety, the covalent conjugate comprising a plurality of carboxylate precursor groups, the carboxylate precursor groups capable of being transformed into carboxylate moieties.

16. (original) The method of claim 15, wherein the covalent conjugate is selected from the group consisting of

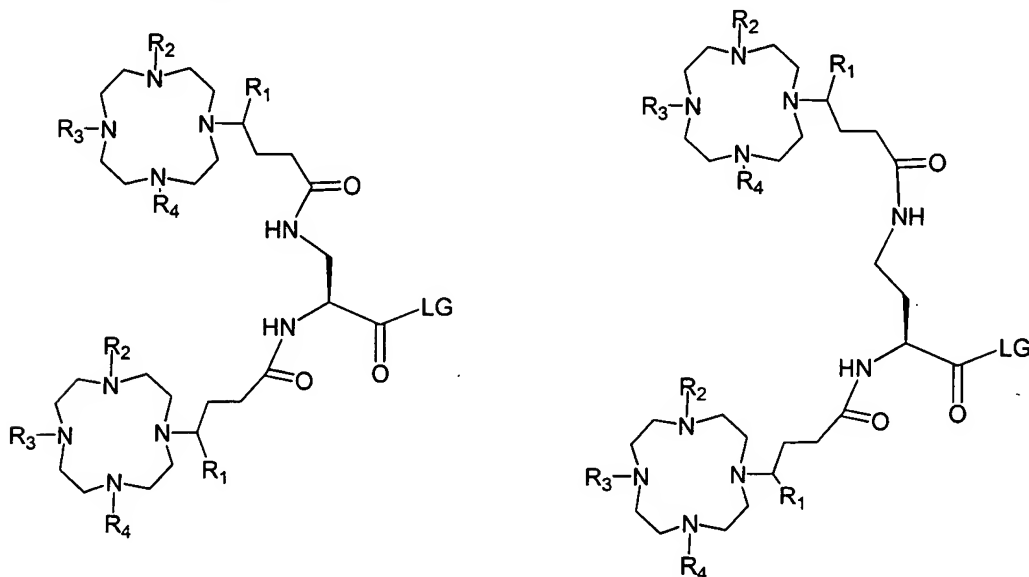


wherein  $n$  is an integer from 1 to 4;

LG is a leaving group selected from the group consisting of  $\text{-OH}$ , activated ester, halide, and anhydride; and

$R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ , and  $R^5$  are independently selected from the group consisting of an acetate moiety, a -Me, -Et, or -t-Bu protected acetate moiety, an acetamide moiety, and an acetoxy moiety.

17. (original) The method of claim 15, wherein the covalent conjugate is selected from the group consisting of:



wherein:

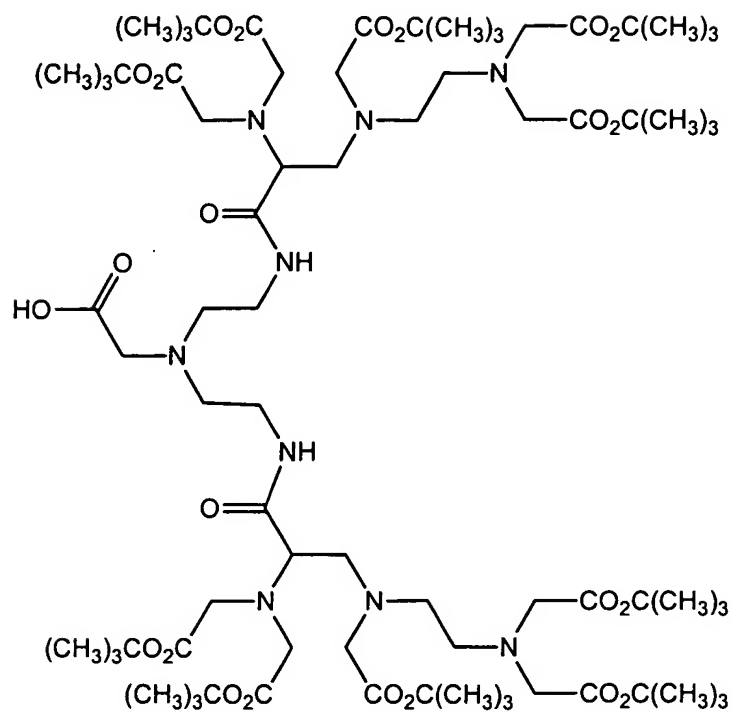
$LG$  is a leaving group selected from the group consisting of  $-OH$ , activated ester, halide, and anhydride; and

$R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are selected from the group consisting of an acetate moiety, a -Me, -Et, or -t-Bu protected acetate moiety, an acetamide moiety, and an acetoxy moiety.

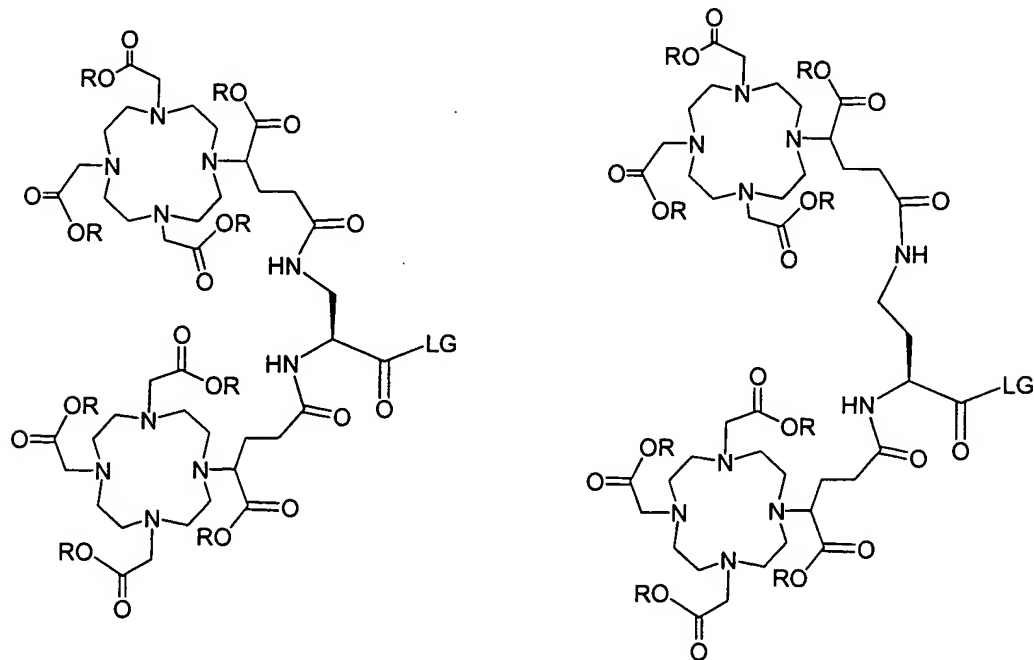
18. (original) The method of claim 15, wherein the covalent conjugate is selected from the group consisting of:

CCOC(=O)CN(CCCOC(=O)C)CCOC(=O)C and CCOC(=O)CN(CCCOC(=O)C)CCOC(=O)C

**Synthon 2:**



19. (original) The method of claim 15, wherein the covalent conjugate is selected from the group consisting of:



wherein:

R is a -tBu group,

LG is a leaving group selected from the group consisting of -OH, activated ester, halide, and anhydride.

20. (original) The method of claim 15, wherein converting the precursor MRI imaging agent to the MR imaging agent comprises:

a) transforming a plurality of the covalent conjugate's carboxylate precursor groups into carboxylate moieties, the carboxylate moieties capable of complexing a paramagnetic metal ion; and

b) complexing a paramagnetic metal ion to the plurality of carboxylate moieties to result in the MR imaging agent.

21. (original) The method of claim 8 or claim 20, wherein the paramagnetic metal ion is selected from the group consisting of: Gd(III), Fe(III), Mn(II and III), Cr(III), Cu(II), Dy(III), Tb(III and IV), Ho(III), Er(III), Pr(III), Eu(II) and Eu(III).

22. (original) The method of claim 21, wherein the paramagnetic metal ion is Gd(III).

23. (original) The method of claim 1, further comprising, prior to step b), reacting a linker-subunit with the N-terminal amine functional group of the peptide to result in a derivatized N-terminal amine functional group of the peptide.

24. - 26. (cancelled).

27. (original) A method of making a MR imaging agent, the method comprising:

- a) covalently binding an amino acid residue to a linker-subunit moiety to form a C-terminal end of a peptide, wherein the linker-subunit moiety is covalently attached to a resin;
- b) synthesizing a peptide on the resin from the covalently bound C-terminal end to an N-terminal residue of the peptide, the N-terminal residue comprising an N-terminal amine functional group;
- c) cleaving the peptide from the resin to produce a peptide having a C-terminal amine functional group;
- d) covalently attaching a linker moiety to the peptide's C-terminal amine functional group and N-terminal amine functional group to form a precursor MR imaging agent; and
- e) converting the precursor MR imaging agent to the MR imaging agent.

28. (original) The method of claim 27, wherein the method further comprises, prior to step c), covalently attaching a linker-subunit moiety to the N-terminal amino functional group to produce a derivatized N-terminal amine functional group.

29. (original) The method of claim 27, wherein converting the precursor MR imaging agent to the MR imaging agent comprises:

- a) reacting the precursor MR imaging agent with a precursor chelate moiety to form a covalent bond between the precursor chelate moiety and the linker moiety of the precursor MR imaging agent, the precursor chelate moiety comprising a plurality of carboxylate precursor groups, the carboxylate precursor groups capable of being transformed into carboxylate moieties;
- b) transforming a plurality of the carboxylate precursor groups of the bound precursor chelate moiety to a plurality of carboxylate moieties, the carboxylate moieties capable of complexing a paramagnetic metal ion; and
- c) complexing a paramagnetic metal ion to the plurality of carboxylate moieties to produce the MR imaging agent.



30. (original) The method of claim 27, wherein the linker moiety is covalently conjugated to a precursor chelate moiety, the covalent conjugate comprising a plurality of carboxylate precursor groups, the carboxylate precursor groups capable of being transformed into carboxylate moieties.

31. (original) The method of claim 30, wherein converting the precursor MRI imaging agent to the MR imaging agent comprises:

- a) transforming a plurality of the covalent conjugate's carboxylate precursor groups into carboxylate moieties, the carboxylate moieties capable of complexing a paramagnetic metal ion; and
- b) complexing a paramagnetic metal ion to the plurality of carboxylate moieties to result in the MR imaging agent.

32. (original) The method of claim 31, wherein the paramagnetic metal ion is selected from the group consisting of: Gd(III), Fe(III), Mn(II and III), Cr(III), Cu(II), Dy(III), Tb(III and IV), Ho(III), Er(III), Pr(III), Eu(II) and Eu(III).

33. (original) The method according to claim 31, wherein the paramagnetic metal ion is Gd(III).

34. – 55. (cancelled).

56. (original) A method for altering the stability of a peptide, the peptide having an N-terminal amine functional group, the method comprising:

- a) reacting the peptide with a linker-subunit moiety to form a peptide having a C-terminal amine functional group; and

b) covalently attaching a linker moiety to the peptide's C-terminal amine functional group and N-terminal amine functional group to form a modified peptide.

57. (original) The method of claim 56, further comprising reacting the modified peptide with a precursor chelate moiety to form a covalent bond between the precursor chelate moiety and the linker moiety of the modified peptide, the precursor chelate moiety comprising a plurality of carboxylate precursor groups, the carboxylate precursor groups capable of being transformed into carboxylate moieties.

58. (original) The method of claim 57, further comprising:

- (a) transforming a plurality of the carboxylate precursor groups of the bound precursor chelate moiety to a plurality of carboxylate moieties, the carboxylate moieties capable of complexing a paramagnetic metal ion; and
- (b) complexing a paramagnetic metal ion to the plurality of carboxylate moieties.

59. (original) The method of claim 57, further comprising assaying the stability of the modified peptide.

60. (original) The method of claim 57, further comprising:

- a) assaying the stability of said unmodified peptide; and
- b) comparing the stability of said modified peptide to the stability of said unmodified peptide.

61. (original) The method of claim 60, wherein the stability of the modified peptide is improved relative to the stability of the unmodified peptide.

62. (original) The method of claim 61, wherein the stability of the modified peptide is improved 10-fold relative to the stability of the unmodified peptide.

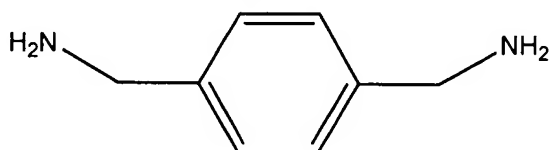
63. (original) The method of claim 61, wherein the stability of the modified peptide is improved 20-fold relative to the stability of the unmodified peptide.
64. (original) The method of claim 61, wherein the stability of the modified peptide is improved 30-fold relative to the stability of the unmodified peptide.
65. (original) The method of claim 59 or claim 60, wherein the stability is assayed using a rat liver homogenate assay.
66. – 67. (cancelled).
68. (original) A method of making an MR imaging agent, the method comprising:  
a) reacting a peptide having an N-terminal amine functional group with a linker-subunit moiety to form a modified peptide having an amine functional group on both its N-terminus and C-terminus; and  
b) converting the modified peptide to the MR imaging agent.
69. (original) A method of making an MR imaging agent, said method comprising:  
a) reacting a peptide having a C-terminal carboxylate functional group with a linker-subunit moiety to form a modified peptide having a carboxylate functional group on both its C-terminus and N-terminus; and  
b) converting the modified peptide to the MR imaging agent.
70. (original) A method of making an MR imaging agent, said method comprising:  
a) covalently binding an amino acid residue to a linker-subunit moiety to form a C-terminal end of a peptide, wherein the linker-subunit moiety is covalently attached to a resin;

- b) synthesizing a peptide on the resin from the covalently bound C-terminal end to an N-terminal residue of the peptide, the N-terminal residue comprising an N-terminal amine functional group;
  - c) cleaving the peptide from the resin to produce a C-terminal amine functional group of the modified peptide;
  - d) converting the modified peptide to the MR imaging agent.
71. (original) The method of claim 68, claim 69, or claim 70, wherein converting the modified peptide to the MR imaging agent comprises covalently attaching a chelate moiety to the modified peptide, wherein the chelate moiety contains a paramagnetic metal ion, to produce the MR imaging agent.
72. (original) The method of claim 71, wherein the paramagnetic metal ion is selected from the group consisting of: Gd(III), Fe(III), Mn(II and III), Cr(III), Cu(II), Dy(III), Tb(III and IV), Ho(III), Er(III), Pr(III), Eu(II) and Eu(III).
73. (original) The method of claim 71, wherein the paramagnetic metal ion is Gd(III).
74. (original) The method of claim 68, claim 69, or claim 70, wherein converting the modified peptide to the MR imaging agent comprises:
- a) covalently linking a linker moiety to a chelate moiety to form a covalent conjugate, wherein the chelate moiety contains a paramagnetic metal ion; and
  - b) reacting the covalent conjugate with the modified peptide to form the MR imaging agent.
75. (original) The method of claim 74, wherein the paramagnetic metal ion is selected from the group consisting of: Gd(III), Fe(III), Mn(II and III), Cr(III), Cu(II), Dy(III), Tb(III and IV), Ho(III), Er(III), Pr(III), Eu(II) and Eu(III).

76. (original) The method of claim 74, wherein the paramagnetic metal ion is Gd(III).
77. (original) The method of claim 56, further comprising reacting the modified peptide with a capping moiety to form a covalent bond between the capping moiety and the linker moiety of the modified peptide.

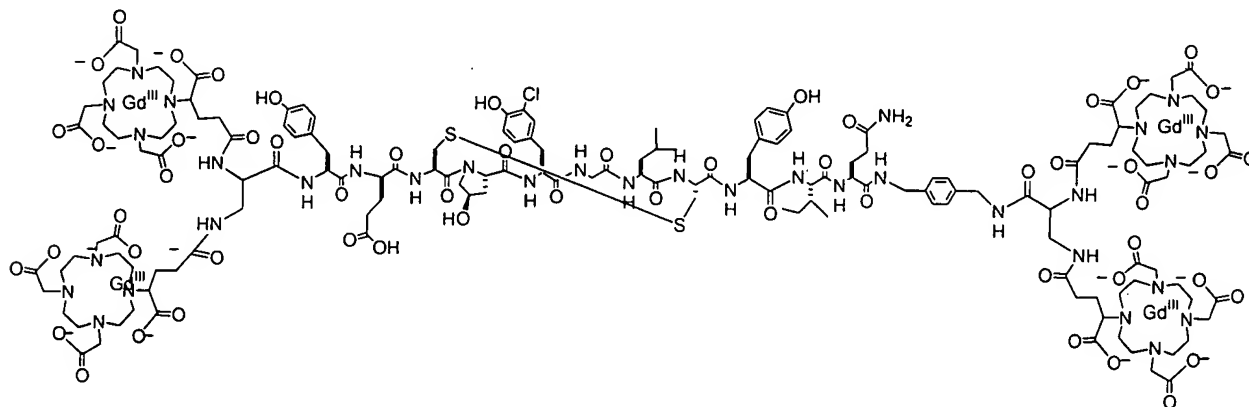
### REMARKS

Claims 1-77 are pending. Applicants have amended the specification to recite priority and related application information. Applicants have also herein amended claim 2 and cancelled claims 24-26, 34-55, and 66-67. Applicants point out that claims 1-23, 27-33, 56-65, and 68-77 correspond to Restriction Group 2, drawn to a method of making an MRI agent using a peptide and the linker subunit moiety:



, as set forth in the Restriction Requirement mailed on January 22, 2004 in parent case U.S. Ser. No. 10/209,183. No new matter has been added. Accordingly, claims 1-23, 27-33, 56-65, and 68-77 are pending.

In the event that the Examiner requests, as in the parent case, that Applicants elect a species within the elected group under 35 U.S.C. § 121, Applicants elect the species of **Structure 36**, shown below:



Claims 1-3, 5-8, 11, 13-15, 17, 19-22, 27, 29-33, 56-65, 68, and 70-76 are drawn to the elected species.

For examination purposes, as in the parent case, Applicants also refer the Examiner to a schematic of Structure 36, attached hereto, depicting its components. Structure 36 includes a modified peptide sequence. On the C-terminal side of the modified peptide (i.e., the right-hand side of Structure 36), the modified peptide is conjugated to one linker-subunit moiety, thereby

forming an amide linkage on the C-terminus of the modified peptide. In turn, the remaining amino moiety of the elected linker-subunit (i.e., the amino moiety in the para position) is conjugated to a linker moiety, also via an amide bond. Next, the linker moiety is conjugated via two amide bonds to two metal chelate complexes. The N-terminus of the modified peptide is not conjugated to a linker-subunit. The N-terminus of the modified peptide forms an amide bond with the same linker moiety as shown on the C-terminal side, which in turn is conjugated via two amide bonds to two of the same metal chelate complexes.

Applicants ask that all claims be examined in view of the amendments to the claims. If necessary, the Examiner is invited to telephone the undersigned attorney if such would expedite prosecution.

Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: \_\_\_\_\_

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